Guide for the Bioequivalence -Module 5

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INTRODUCTION

Two medicinal products containing the **same active substance** are considered bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and their bioavailabilities (rate and extent) after administration in the **same molar dose** lie within acceptable predefined limits. These limits are set to ensure comparable *in vivo* performance, i.e. similarity in terms of safety and efficacy (EMEA).

A **generic medicinal product** is a product which has the same qualitative and quantitative composition in active substances and the same pharmaceutical form as the reference medicinal product (list of References can be found on FDA or EMEA sites), and whose bioequivalence with the reference medicinal product has been demonstrated by appropriate bioavailability studies.

The different salts, esters, ethers, isomers, mixtures of isomers, complexes or derivatives of an active substance are considered to be the same active substance, unless they differ significantly in properties with regard to safety and/or efficacy. Furthermore, the various immediate-release oral pharmaceutical forms shall be considered to be one and the same pharmaceutical form.

Although the concept of bioequivalence possibly could be considered applicable for herbal medicinal products, the general principles outlined in this guideline are not applicable to herbal medicinal products, for which active constituents are less well defined than for chemical entities.

Based on these definitions, MOPH will not accept any application for a generic drug:
1. if the bioequivalence study is not aimed to compare the generic (test) to a brand (reference) containing the same active substance(s).
2. if the reference brand drug is not registered in any of the referenced countries (approved by the technical committee of the MOPH as referenced countries).

In addition, the bioequivalence study should not be prepared by the same pharmaceutical industry producing the generic drug. An independent CRO should realize the study (all sections of the study).

Standard design

If two formulations are compared, a randomized, two-period, two-sequence, single dose, crossover design is recommended. The treatment periods should be separated by a wash out period sufficient to ensure that drug concentrations are below the lower limit of bioanalytical quantification in all subjects at the beginning of the second period. Normally at least 5 elimination half-lives are necessary to achieve this (7 half-lives are required to eliminate 99 % of the drug).

Under certain circumstances an alternative well-established designs could be considered if the company provide a scientifically reasonable study design, statistical analyses.

If applicable, both sex (male and females) should be included in the study design.

Choosing the reference batch

Unless otherwise justified, the assayed content of the batch used as test product should not differ more than 5% from that of the batch used as reference product determined with the test procedure proposed for routine quality testing of the test product. The Applicant should document how a representative batch of the reference product with regards to dissolution and assay content has been selected. It is advisable to investigate more than one single batch of the reference product when selecting reference product batch for the bioequivalence study.

Choosing the test product units to be tested in the BE:

The test product used in the study should be representative of the product to be marketed and this should be discussed and justified by the applicant.

For example, for oral solid forms for systemic action:

a) The test product should usually originate from a batch of at least 1/10 of production scale or 100,000 units, whichever is greater, unless otherwise justified.

b) The production of batches used should provide a high level of assurance that the product and process will be feasible on an industrial scale. In case of a production batch smaller than 100,000 units, a full production batch will be required.

c) The characterization and specification of critical quality attributes of the drug product, such as dissolution, should be established from the test batch, i.e. the clinical batch for which bioequivalence has been demonstrated.

d) Samples of the product from additional pilot and/or full scale production batches, submitted to support the application, should be compared with those of the bioequivalence study test batch, and should show similar *in vitro* dissolution profiles when employing suitable dissolution test conditions.

Comparative dissolution profile testing should be undertaken on the first three production batches.

If full scale production batches are not available at the time of submission, the applicant should not market a batch until comparative dissolution profile testing has been completed.

The results should be provided at a Competent Authority's request or if the dissolution profiles are not similar together with proposed action to be taken.

For other immediate release pharmaceutical forms for systemic action, justification of the representative nature of the test batch should be similarly established.

Which Dose to be tested?

If several strengths of a test product are applied for, it may be sufficient to establish bioequivalence at only one **(in general the highest one <u>unless justified</u>)** or two strengths, depending on the proportionality in composition between the different strengths and other product related issues. The strength(s) to evaluate depends on the linearity in pharmacokinetics of the active substance.

In case of non-linear pharmacokinetics (i.e. not proportional increase in AUC with increased dose) there may be a difference between different strengths in the sensitivity to detect potential differences between formulations. In the context of this guideline, pharmacokinetics is considered to be linear if the difference in dose-adjusted mean AUCs is no more than 25% when comparing the studied strength (or strength in the planned bioequivalence study) and the strength(s) for which a waiver is considered.

In order to assess linearity, the applicant should consider all data available in the public domain with regard to the dose proportionality and review the data critically. Assessment of linearity will consider whether differences in dose-adjusted AUC meet a criterion of $\pm 25\%$.

If bioequivalence has been demonstrated at the strength(s) that are most sensitive to detect a potential difference between products, *in vivo* bioequivalence studies for the other strength(s) can be waived.

<u>BE report should include the following documents</u>

When you submit the BE application to the Lebanese MOPH, make sure that you provide the following documents/items:

- Letter from the company (signed and dated) explaining your application and describing all submitted documents.
- BE application (attached with this requirement: section I to Section VI). All questions in each section should be answered and completed in order to review the application.
- In addition to a soft copy, a CD including the complete BE study should be submitted.
- Copy of the signed informed consents **for all subjects** enrolled in the study.
- Copy of the CRF **for all subjects** enrolled in the study.
- Copy of subjects' chromatograms realized in analytical section.
- Copy of all investigators CV
- CD including **raw data** (as Excel sheet) for all analytical validation method.
- CD including **raw data** (as Excel sheet) for plasma drug concentration at each time point for each subject enrolled in the study.

Raw data on Excel sheets are required because the Lebanese MOPH has the right to repeat any analysis/statistical analysis to validate the data presented in the BE study.

<u>Notes</u>**:**
1- Any bad quality scanned document will be refused.
2- Correct scale for all individual plasma drug concentration *versus* time plots should be used and **no more than 2 plots per page** is allowed.
3- Plasma drug concentration *versus* time plots should be provided in both **linear and semi-logarithmic** scale.

Section I – Generalities

Sponsor should provide answers to all questions below and attach any required document:

1. Study Title:
2. Name of the active ingredient(s):
3. Manufacturer/Sponsor:
4. Therapeutic class:
5. Information about the Test Product
    a. Brand Name:
    b. Dosage:
    c. Pharmaceutical form:
    d. Manufacturer:
    e. Batch number:
    f. Manufacturing date:
    g. Expiry date:
6. Information about the Reference Product
    a. Brand Name:
    b. Dosage:
    c. Pharmaceutical form:
    d. Manufacturer:
    e. Batch number:
    f. Manufacturing date:
    g. Expiry date:
7. Provide evidence showing that it is a reference product according to FDA or EMEA lists.
8. Dose used in the BE study:
9. Information about the CRO
    a. Name:
    b. Country:
    c. Address:
10. CRO study sites:
    a. Clinical (hospital):
    b. Clinical/medical Laboratory (for screening examination):
    c. Analytical Facility:
    d. Pharmacokinetic studies:
    e. Statistics:
11. Protocol study N°:
12. Sponsor should provide information showing if the study was done according to FDA/EMA/others guidelines
13. Sponsor should provide the certificate of analysis for both reference and test products.

Section II – Clinical Part

Sponsor should provide answers to all questions below and attach any required document:

1. Sponsor should provide a copy of the study protocol.
2. Sponsor should provide any amendment to the study protocol (if available).
3. Sponsor should include a copy of the informed consent form
   Note: signed informed consent for all participants should be attached in BE report.
4. Sponsor should provide the IRB protocol approval.
   Note: Sponsor should attach the IRB approval document (dated with all committee members signatures).
5. Official certificates of GCP and GLP compliances should be provided in the BE report.
   Note: Date of certificates should fit the study period.
6. Quality assurance (QA) audits performed by the CRO with dates and signatures.
7. Official authorization (certificate) for laboratory where routine lab analysis had been done.
8. GMP (for the manufacturer).
9. Sponsor should provide the following dates/period:
   a. Informed Consent was signed by applicants on:
   b. Date of the screening examination:
   c. Period I started on:
   d. Period I ended on:
   e. Period II started on:
   f. Period II ended on:
   g. First blood sample was taken on:
   h. Last blood sample was taken on:
   i. Study report released on:
10. Sponsor should provide the study duration:
   a. For the clinical part:
   b. For the bioanalytical part:
   c. For the statistical part:
11. Wash out period:
   Note: it should be at least 5 $t_{1/2}$ of the active ingredient(s).
12. Sponsor should indicate the drug half-life.
13. Sponsor should provide the sample size (number of subjects included and number of subjects who completed both periods of the study). Sponsor **should detail the method used to calculate the minimum number of subjects** required to be enrolled in the study. Number of subjects completed both periods should not be less than the calculated minimum number of subjects required.
14. Number of blood sample per subject.
15. Sponsor should indicate the time period for each blood sample.
16. Sponsor should provide the total volume of blood drawn per subject and describe it.
17. Sponsor should provide the anticoagulant used in blood test tube.
18. Sponsor should indicate how samples were stored (at clinical site and analytical site) and transported to the analytical site. (i.e. samples were stored on ice/4 °C/-20 °C/-80 °C/etc …)

19. All screening examination data and individual Case Report Form should be provided.

20. Sponsor should provide a table with all adverse events and discuss it.

Section III – *In vitro* Dissolution Profile

Sponsor should provide answers to all questions below and attach any required document:

1. Dates (start and end of analysis)

2. Sponsor should provide dissolution data **as tables and figures** for each of the used medium.

3. Sponsor should provide the number of dissolution units used (for test and reference drugs). The dissolution profiles can be compared only when number of **dissolution units used are equal to or greater than 12**.

4. Sponsor should also provide sampling time, method of assay of the active ingredient dissolved, limits used.

5. Sponsor should provide the composition of the medium used (3 media are required). For immediate release forms, the 3 media are **HCl 0.1N, Acetate buffer pH 4.5 and phosphate buffer pH 6.8. Any changes in the medium composition should be justified.**

6. For each medium used you have to provide the following:
  a. Medium composition and pH:
  b. Apparatus used:
  c. Speed (rpm):
  d. Temperature:
  e. Volume used:
  f. Duration:
  g. Difference factor (f1) =
  h. Similarity factor (f2) =

<u>Notes:</u>

1- Similarity factor of 50 – 100 ensures sameness of two products. i.e. *f2* value between 50 and 100 suggests that the two dissolution profiles are similar.

2- Difference factor of 0 – 15 ensures minor difference between two products. i.e. *f1* value between 0 and 15 is required.

3- For rapid dissolving products, that may dissolve 85% in 15 minutes, calculation of *f1* and *f2* is not required.

Section IV – Analytical Validation method

Reminder: <u>Raw data</u> should be saved on excel sheet and provided to MOPH on separated CD.

Sponsor should provide answers to all questions below and attach any required document:

1. Sample preparation and drug/metabolite extraction method used
2. The analytical method used to quantify the analyte.
3. Analyte (drug or metabolite) measured in plasma/urine.
4. Certificate of analysis for the reference standards used in the analysis
5. The internal standard used. **It's obligatory to use an internal standard.**
6. Provide information about the biological matrix used in the preparation of the standard curve.
7. Method used should be validated for the following parameters:
    a. Linearity: provide the linearity zone, standard curve equation and R^2.
      No sample could be used in the BE calculations if outside the linearity zone tested.
    b. Recovery (extraction yield) for drug (metabolite) and for internal standard.
    Note: The **recovery** of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. **Recovery of the analyte need not be 100%,** but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery.
    c. Inter-day Accuracy
    d. Intra-day Accuracy
    Note: The **accuracy** of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The mean value **should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%.** The deviation of the mean from the true value serves as the measure of accuracy.
    e. Inter-day Precision
    f. Intra-day Precision
    Note: The **precision** of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level **should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV.** Precision is further

subdivided into within-run, intra-batch precision or repeatability, which assesses precision during a single analytical run, and between-run, interbatch precision or repeatability, which measures precision with time, and may involve different analysts, equipment, reagents, and laboratories.

g. Calculated concentrations of calibrate samples of the active ingredient in human plasma

h. Matrix effect

i. Anticoagulant (present in the sampling tubes) effect

j. Short term Stability of active ingredient and internal standard in **stock solutions** at different temperatures (such as room temperature, -4 °C, -20 °C).

k. Short term Stability of active ingredient and internal standard in **human plasma** at different temperatures (such as room temperature, -4 °C, -20 °C).

l. Long term stability of active ingredient and internal standard in **stock solutions** at different temperatures (such as room temperature, -4 °C, -20 °C). **This section should cover the period between period I and the end of analytical measurements.**

m. Long term stability of active ingredient and internal standard in **human plasma** at different temperatures (such as room temperature, -4 °C, -20 °C). **This section should cover the period between period I and the end of analytical measurements.**

n. Freeze/thaw stability of active ingredient.
Sponsor should provide stability for n cycles of freeze/thaw at specific temperature.

o. Specificity in the presence of different compounds added.

p. Robustness.

q. Sensitivity: the lowest limit of detection (LLOD), the lowest limit of quantification (LLOQ)
Note: **LLOQ** is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision. The LLOQ should be established using at least five samples independent of standards and determining the coefficient of variation and/or appropriate confidence interval. The LLOQ should serve as the lowest concentration on the standard curve and **should not be confused with the limit of detection** and/or the low QC sample. The highest standard will define the upper limit of quantification (ULOQ) of an analytical method.

r. Concentration of the quality control samples used with their CV: minimum 3 concentrations:
    r.1. Low QC
    r.2. Medium QC (one or two)
    r.3. High QC

8. A minimum of 20% of all subjects analytical spectrums should be provided in the BE report.

Section V – Pharmacokinetic Analysis Section

1. Sponsor should provide on excel sheet (on attached CD) all raw data related to plasma concentration for all subjects and at all time points. No scanned sheet is allowed.

2. Sponsor should provide the mean plasma concentration vs. time plot in both linear and semi-logarithmic scale (with SEM/SD error bars on each point).

3. Sponsor should provide individual plasma concentration vs. time plot in both linear and semi-logarithmic scale for all subjects.

4. Calculation of pharmacokinetic parameters: $AUC_{0 \to t}$, $AUC_{0 \to \infty}$, C_{max}, T_{max}, $t_{1/2}$, K_e, etc …
Non-compartmental methods should be used for determination of pharmacokinetic parameters in bioequivalence studies. The use of compartmental methods for the estimation of parameters is not acceptable.

    a. Actual time of sampling should be used in the estimation of the pharmacokinetic parameters.
    b. In studies to determine bioequivalence **after a single dose**, $AUC(0 \to t)$, $AUC(0 \to \infty)$, residual area, C_{max} and t_{max} should be determined.
    c. **In studies with a sampling period of 72 h**, and where the concentration at 72 h is quantifiable, $AUC(0 \to \infty)$ and residual area do not need to be reported; it is sufficient to report AUC truncated at 72h, $AUC(0 \to 72h)$. In addition, C_{max} and T_{max} should be calculated.
    d. Additional parameters that may be reported include the terminal rate constant, K_e, and $t_{1/2}$.
    e. In studies to determine bioequivalence for immediate release formulations at steady state, $AUC(0-|)$, $C_{max,ss}$, and $t_{max,ss}$ should be determined.
    f. When using **urinary data**, $Ae(0 \to t)$ and, if applicable, R_{max} should be determined.

5. Sponsor should provide the software used to do the calculation and statistical analysis.

6. Sponsor should complete the following table for the Log-transformed Test/Reference ratios:

Parameters	Cmax	$AUC_{0 \to t}$	$AUC_{0 \to \infty}$
Criteria	**80 – 125 %**	**80 – 125 %**	**80 – 125 %**
Mean			
90% Confidence Interval			
Intra-subject variability			

7. In case the criteria are different than 80 – 125%, the sponsor should provide detailed explanation and provide additional references that allow such modification. **Any unjustified wideness of these criteria will be rejected. Any intra-subject variability should be discussed according to literature.**

Section VI – Statistical Analysis Section

1. Sponsor should provide the software used to do the ANOVA analysis.

2. Sponsor should complete the table below with corresponding p values.

Source	p values		
	Cmax	**$AUC_{0 \to t}$**	**$AUC_{0 \to \infty}$**
Period			
Subject (seq)			
Formulation			
Sequence			

3. Sponsor should provide explanation or additionnal tests in case any p-value in the table above is < 0.05 (Statistically significant). BE study will be rejected in absence of any explanation/justification.